Biochip Multi-function Signal Generator

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Abstract

The University of Canterbury recently developed a biochip, or "lab-on-a-chip" platform, which is used for the diagnosis and fast analysis of biological samples [1]. The biochip uses dielectrophoresis (DEP) to manipulate cells and other small particles [2]; this requires a.c. waveforms with well-defined frequency and amplitude. Hence, a dual-channel signal generator, capable of generating sine, square and triangle waveforms at frequencies up to 20 MHz and amplitudes up to 20 V_{pp}, was designed and built. A series of experiments using the signal generator and biochip demonstrated the effects of positive and negative DEP on polystyrene beads.

Keywords: biochip, lab-on-a-chip, dielectrophoresis, signal generator.

1 Introduction

The University of Canterbury has developed a platform to analyse and manipulate micron-scale biological samples, including living cells [1]. This biochip platform relies on voltage waveforms applied across several electrodes to control small particles [2]. To date these voltage waveforms have been supplied by several inter-connected function generators, each providing a different waveform. A single signal generator, capable of supplying all of the desired waveforms through a number of independent channels, was desired to standardise the signals that are delivered to the biochip.

For this reason, a multi-purpose signal generator was developed from the component level up, to drive lab-on-a-chip systems built using biochip technology. The signal generator had two independent output channels and was capable of delivering sine, square and triangle waveforms with frequencies and amplitudes up to 20 MHz and 20 V_{pp}, respectively. Hardware support for two additional independent arbitrary waveform channels was also included.

The signal generator was used to power the biochip in a series of experiments that demonstrated both positive and negative dielectrophoresis (DEP) acting on ~10 µm polystyrene beads. These experiments are discussed in this report, along with a description of the design and construction of the signal generator.

2 Specifications

The signal generator had to be able to deliver signals with a wide range of frequencies at variable amplitude, so that it could be used in a variety of biochip-based experiments. Various waveforms, such as sine, square and triangle, were required. The signal generator needed at least two independent outputs, with selectable impedance. These design parameters are summarised in Table 1 [3].

Table 1: Signal Generator Design Specifications.

Parameter	Value
Number of Channels	≥ 2, independent
Output Waveform	Sine, Square, Triangle and Arbitrary
Frequency Range	near d.c. – 20 MHz
Duty Cycle	50%, fixed
Amplitude Range	0 V_{pp} – 25 V_{pp}
Offset Voltage	0 V, fixed
Output Impedance	50 Ω / 300 Ω, selectable

3 Theory of Operation

Phenomena, such as DEP, that are experienced by biological cells and small particles are dependent on the frequency, intensity and flux of the electric field that surrounds them. Therefore, the basic approach used was to build a signal generator that was capable of delivering output signals with a wide range of frequency and amplitude, thus allowing variable dielectrophoretic forces to be applied to selected particles. An explanation of these dielectrophoretic forces is given below.

Dielectrophoresis is the name given to the motion caused by a force acting on a neutral molecule or particle suspended in a non-uniform electric field [4], [5]. The dielectrophoretic force depends on a number of factors, including the polarisability and dimensions of the particle in question, the polarisability of the surrounding medium and the degree of non-uniformity of the electric field.

The relationships between these factors as they apply to a spherical particle are:

$$F_{dep} = 2\pi\varepsilon_1 R^3 \,\mathrm{Re}[K(\omega)]\nabla E_0{}^2 \qquad (1)$$

$$K(\omega) = \frac{\varepsilon_2^* - \varepsilon_1^*}{\varepsilon_2^* + 2\varepsilon_1^*} \qquad (2)$$

where: F_{dep} = dielectrophoretic force, N; ε_1 = permittivity of the medium surrounding the particle, F/m; R = radius of the spherical particle, m; $K(\omega)$ = Clausius-Mossotti function, defined in Eqn. 2; ε_2 = permittivity of the particle, F/m; ∇ = del operator; and E_0 = electric field, V/m [6].

Equation 1 is valid provided the electric field does not change significantly over the particle length, and shows that depending on the relative magnitudes of ε_1 and ε_2, the dielectrophoretic force developed can be either positive (acting towards areas of high field density) or negative (acting towards areas of low field density). The dependence of a particle's permittivity on the frequency of the surrounding a.c. electric field implies that different particles experience stronger or weaker dielectrophoretic forces depending on the frequency of the applied field. This means, for example, that a particle may experience a positive dielectrophoretic force at one frequency and a negative force at another frequency. The difference in permittivity between different types of particles also allows the separation of heterogeneous mixtures of particles into homogenous populations [7], as well as the geographical isolation of some particles while others move about unimpeded.

4 Design and Fabrication

The design of the signal generator can be viewed as inter-connected groups of small sub-circuits, which are grouped by the type of function that they perform. The main groups, shown in Fig. 1, were built around waveform generation, amplification, output sensing and the user/machine interface. The entire system was implemented on two printed circuit boards (PCBs) housed inside an aluminium case, shown in Fig. 2.

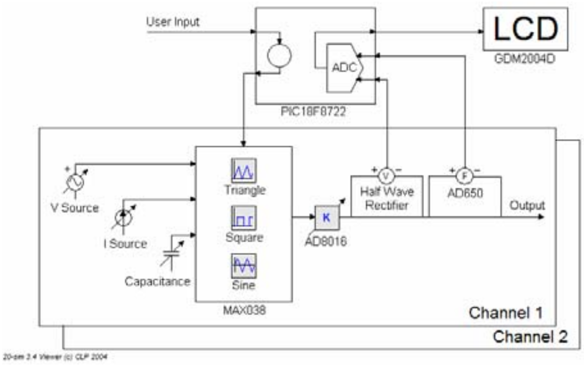

Figure 1: System block diagram.

Figure 2: A photograph of the multi-function signal generator.

4.1 Waveform Generation

Sine, square and triangle signals were produced using two Maxim MAX038 high frequency waveform generators. Two TTL inputs per chip controlled the shape of the output waveform, while the frequency was controlled via a capacitor and current supply. Since the required current was relatively small (2 µA – 750 µA), a full current supply could be replaced by a 2.5 V reference voltage and a resistor-divider network. The corresponding equation defining the relationship between the input voltage, the resistance, the discharge capacitance and the output frequency is $F_0 = V_{\mathrm{REF}} / R_{\mathrm{IN}}C$ [8]. Fine control of the frequency was handled by a small (±2.3 V) voltage applied to the MAX038's $\mathrm{F_{ADJ}}$ pin. The amplitude of the output waveforms was fixed at 2 V_{pp} and although the MAX038 could vary the output duty cycle between 15% and 85%, it was also fixed at 5a0%.

Despite the nominal range of input currents spanning only two orders of magnitude, output frequencies spanning four orders of magnitude, from 2 kHz to 20 MHz, were achieved. This was made possible by attaching a dual-pole, dual-throw (DPDT) switch, connected to two capacitors of different values, to each MAX038. Hence, two frequency ranges were created on each waveform chip, one spanning 2.0 kHz to 2.0 MHz, the other 26 kHz to 20 MHz. The capacitors used to create these ranges were 470 pF and 22 pF, respectively.

4.2 Amplification and Output Impedance

AD8016 dual xDSL line drivers from Analog Devices were used to amplify the 2 V_{pp} signals from the waveform generators up to a maximum of ~20 V_{pp}. The signals were attenuated by a variable resistor-divider network before they reached the line drivers, which were configured with a fixed gain of 13.2 x. This arrangement produced a maximum theoretical output amplitude of 24 V_{pp}. The resistors in the feedback network were chosen to avoid amplifier instability; following guidelines in the data sheet [9], R_1 and R_2 were set to 82 Ω and 1 kΩ, respectively.

DPDT switches, similar to the ones used with the waveform generators, were used to give two levels of output impedance. 51 Ω and 300 Ω resistors were used to give selectable output impedance of 64 Ω and 312 Ω at d.c., these values were ~20 Ω higher at 20 MHz. Despite this, attenuation into a 50 Ω load was less than 10% at frequencies up to ~15 MHz when 50 Ω_{nom} output impedance was selected. Coaxial BNC output connectors were used to ensure compatibility with a wide range of test equipment.

4.3 Signal Measurement and the User Interface

A PIC18F8722 microcontroller from Microchip Technology, Inc. was used to collect data on the output signals and handle some user input. Two of the microcontroller's 16 analogue input pins were used in combination with its built-in 10-bit analogue-to-digital converter (ADC) and a resistor-divider network to measure the output voltage of each waveform. Two more pins were used in a similar way to measure the voltage generated by two AD650 frequency-to-voltage conversion chips, which were connected to the signal generator's outputs. The signals controlling the MAX038's waveform selection pins were routed through the microcontroller, providing user-controlled input to the microcontroller that was useful during software debugging.

A Xiamen Ocular GDM2004D 20 x 4 character liquid crystal display (LCD), based on the Hitachi HD44780 chipset, was used to display information about the output waveforms to the user. It was set up to show the channel number, frequency and amplitude of each waveform simultaneously. The LCD was controlled by one of the microcontroller's general-purpose I/O banks and was configured in 4-bit write mode to simplify its operation. For the same reason, the contrast of the display was fixed by an internal potentiometer that was configured during assembly and the LCD backlight was permanently lit while the signal generator was running.

Analogue potentiometers were used to give the user accurate control of the output waveform. Two frequency dials, an amplitude dial and waveform-, frequency range- and impedance-selection switches were used to give a powerful, user-friendly interface. Analogue controls were favoured over digital equivalents for two reasons: firstly, they imposed no hard limit on the resolution of the output waveform; secondly, they were easier to interface directly to the waveform generation chips and amplifiers. In many cases, digital controls needed to be translated by the microcontroller before they could be connected to the underlying hardware. To simplify the assembly process, the user controls were designed to easily plug in and out of connectors on the PCBs using a wiring harness.

Lastly, a USB interface was included, implemented via an FTDI USB-to-serial converter (FT232BL) connected to one of the microcontroller's two universal synchronous / asynchronous receiver / transmitter (USART) units. This interface allowed waveform data to be transmitted to a personal computer, effectively automating the data recording process. Another benefit was that the signal generator's software could be updated using a personal computer without removing any of the aluminium casing. The USB connection operated at 9600 baud, sending packets of eight data bits, one stop bit and no parity-checking bits.

4.4 PCB Layout and Fabrication

Circuit blocks critical to the success of the project were laid out on a two-sided, 152 mm x 104 mm (6.0" x 4.1") PCB. These blocks included the waveform generation chips, their amplifiers, the PIC microcontroller and USB chip, as well as several op-amps and current sensors, used to measure the current flowing in each channel. The majority of the control panel wiring harness was also connected to this board. Surface mounted resistors and capacitors with 0805 footprints and 5% tolerances were used in the majority of cases; however, where more precise values were required components with 0603 footprints and 1% tolerances were used. A small number of 1206 components were used to allow traces to pass under them without being routed to a different layer. Due to the fine pitch of the microcontroller and amplifier pins, the PCB could not be etched and had to be milled by an LPKF Protomat 100 milling machine. This machine was capable of milling boards with a minimum trace/space size of 0.1 mm / 0.1 mm (< 4 mil / 4 mil); the tightest dimensions on the board were 0.2 mm / 0.2 mm (< 8 mil / 8 mil). After milling, nail polish was used to insulate low-clearance parts of the board to prevent short circuits from forming during the soldering process.

A second board was made to accommodate the power supply connections and an auxiliary custom waveform generation system. This system was designed to provide two independent, variable-frequency custom waveforms and was made up of a 200,000 gate Xilinx Spartan II-E FPGA, a THS8135 tri-channel digital-to-analogue converter (DAC) and a CDC960 200MHz clock generator. Two amplifiers, similar to those used on the first board, were also included. The board was connected directly to an ATX power supply and used LP3964 regulators from National Instruments to isolate, regulate and step down the various supply voltages (1.8 V digital, 3.3 V analogue and digital, ±5 V analogue and digital and ±12 V analogue) used by the signal generator. The auxiliary board was larger than the main board at 160 mm x 136 mm (6.3" x 5.4"), with the same minimum trace/space ratio of 0.2 mm / 0.2 mm.

5 Discussion

After fabrication, the signal generator's performance was measured and compared to a set of expected values and benchmarks. Voltage waveforms, showing the signal generator's maximum output amplitude and frequency, were captured and used to show the independence of the signal generator's output channels. Once the signal generator's capabilities were known, a number of experiments were completed to demonstrate the effects of DEP.

5.1 Measured Performance

Table 2 shows the specifications met by the completed signal generator. These parameters were measured using an oscilloscope and a multimeter. The broad frequency range and fixed offset voltage stand out, as does the selectable output impedance. Disappointingly, the required maximum amplitude was not met; this was due to a fault in the ATX power supply that was used. The power supply delivered only ~-8 V on its -12 V rail, regardless of load. If it had met its specification [10] and delivered -12 V ± 10%, the maximum amplitude would have been closer to the ~25 V_{pp} called for. Finally, arbitrary output waveforms were not supported by the signal generator's software; however, all of the necessary hardware was included on the auxiliary PCB.

Table 2: Signal generator measured performance.

Parameter	Realised
Number of Channels	2, independent
Output Waveform	Sine, Square and Triangle
Frequency Range	Low: 1.9 kHz – 2.0 MHz High: 26.0 kHz – 20.7 MHz
Duty Cycle	50%, fixed
Amplitude Range	0 V_{pp} – 18.3 V_{pp} (no clipping) 18.3 V_{pp} – 19.8 V_{pp} (clipping)
Offset Voltage	0 V, fixed
Output Impedance	64 Ω / 312 Ω, selectable

Figure 3 shows the three different output waveforms from the signal generator at arbitrary frequency and amplitude. The signal spectrum for each waveform is also shown. The same waveforms and spectra at maximum distortion-free amplitude are shown in Fig. 4, with the maximum output frequency shown in Fig. 5. Figure 6 shows the independence of channel two with respect to channel one.

5.2 Application

After its fabrication and testing, the signal generator was used in a series of DEP experiments using the biochip, which demonstrated basic dielectrophoretic principles and verified some of the associated theory. In the experiments, one of the signal generator's output channels was connected to the biochip via a BNC coaxial connector and alligator clips. The biochip was placed under a microscope and had a drop of water, laced with micron-scale polystyrene beads, placed on its surface. The microscope, an Olympus BX60 coupled to an Olympus DX30 digital camera, was connected to a personal computer to facilitate data capture and recording; a T-connector and BNC cable were used to connect the signal generator to an oscilloscope for the same reason.

One of the series of experiments performed used parallel comb-shaped electrodes to create regions of high and low electric field flux density. Positive DEP was demonstrated by trapping beads in regions of high flux, such as between opposing electrode-comb 'teeth' and at the interface between electrodes and the surrounding insulating substrate of the biochip (Fig. 7). Sine waveforms of ~12 V_{pp} amplitude and ~80 kHz frequency were used to achieve this. Negative DEP was shown by changing the frequency of the applied waveform to ~1.5 MHz; the beads then congregated at points farthest from the electrode/insulator boundaries (Fig. 8). Due to its variable waveform shape capabilities, the signal generator was also able to show that altering the applied waveform between sine, square and triangle shapes had no visible impact on the type of DEP observed or the rate at which beads became ordered according to the electric field flux lines.

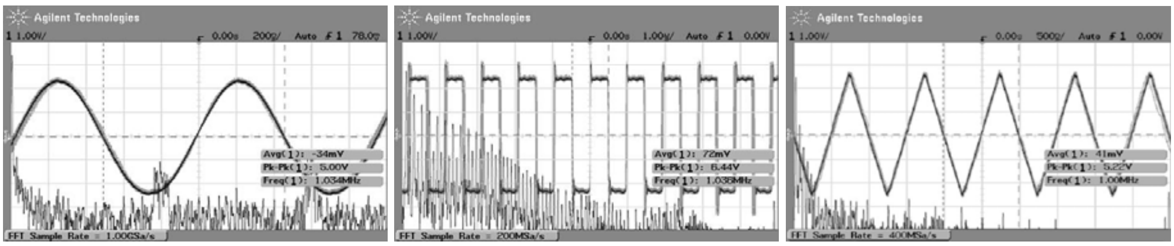

Figure 3: Sine, square and triangle waveforms (heavy traces) at 1MHz and 5 V_{pp}, with the fast Fourier transforms (FFTs) of the waveforms shown (light traces).

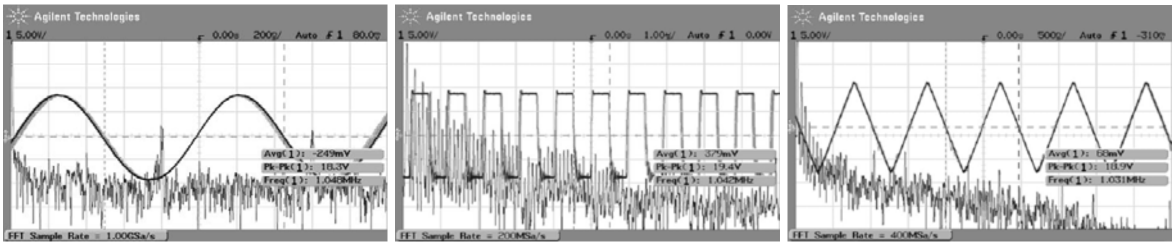

Figure 4: 1MHz waveforms (heavy traces) with maximum clean amplitude between 18.3 V_{pp} and 19.4 V_{pp}. FFTs of these waveforms are also shown (light traces).

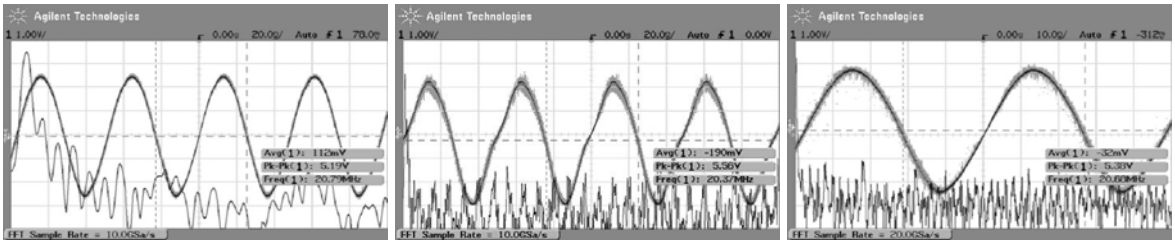

Figure 5: Output waveforms (heavy traces) at maximum frequency of 20.7 MHz and amplitude of 5 V_{pp}. The FFTs of the waveforms (light traces) show the signal degradation experienced at high frequencies.

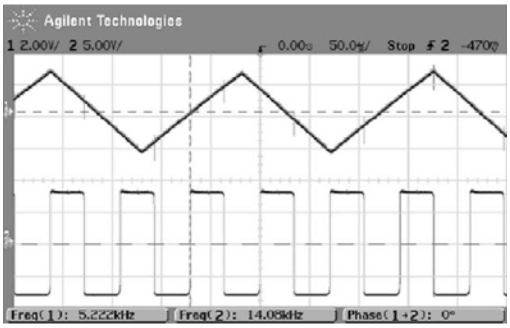

Figure 6: Top: Output channel one. Bottom: Output channel two. Both waveforms have different shapes, frequencies and amplitudes.

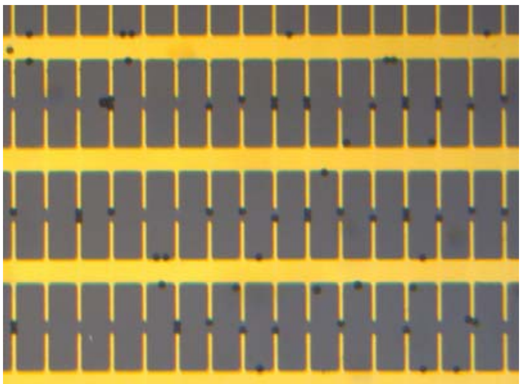

Figure 7: Beads (dark spheres) gathered at regions of high field density demonstrate positive DEP.

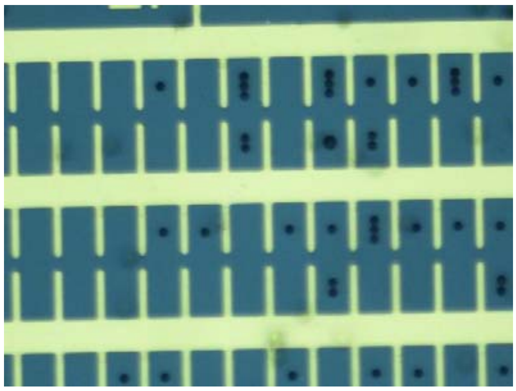

Figure 8: Beads clustered away from conductor-insulator junctions demonstrate negative DEP.

6 Conclusion

A 20 MHz, dual-channel signal generator was designed for use with the University of Canterbury's biochip technology, as well as a range of other standard equipment. The signal generator contained two independent channels, capable of delivering sine, square and triangle waveforms at amplitudes up to 20 V_{pp}, with output impedances of either 50 Ω or 300 Ω. A user-friendly interface based around analogue controls, as well as a large, easy to read LCD panel, completed the design.

An oscilloscope was used to characterise the signal generator's output waveforms. These were of a high quality at low- and mid-range frequencies but deteriorated at high frequencies. Although this was not desirable, it was an expected part of the waveform generation devices' performance and was documented in their data sheet [8]. Despite this marginal drawback, the signal generator met or exceeded the majority of the required specifications and was able to induce positive and negative DEP on polystyrene beads. Furthermore, the shape of the applied waveform generated by the signal generator was shown to have only negligible effect on the dielectrophoretic forces.

One of the possible additions to the signal generator system is an extended embedded software suite that would support the signal generator's two custom waveform channels. The required software would comprise a memory block inside the auxiliary board's FPGA, as well as a method of cycling through the memory locations at a high frequency, supplied by the CDC960 clock generator. No additional hardware would be required, since all the necessary components were installed during assembly and were connected to the case as outputs three and four.

Another possible extension involves building a phase splitter for use with the signal generator. This would allow two or four identical signals, either 90°, 180° or 270° out of phase, to be applied to the biochip. As well as allowing electrorotation experiments, this would make travelling-wave dielectrophoresis possible [11]. A rudimentary phase splitter could be added to the signal generator in its current configuration by linking its MAX038 modules via their internal phase-detection circuitry.

7 Acknowledgements

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8 References

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